HUMAN BREAST MILK LIPID MIMETIC AS DIETARY SUPPLEMENT

Field of the Invention

The present invention refers to the field of infant nutritional foods. More specifically, the present invention describes novel fat compositions which are components in the preparation of fat blends and infant formulas, as well as the process of producing the same.

Background of the Invention

All publications mentioned throughout this application are fully incorporated herein by reference, including all references cited therein.

Lipids in general are the building blocks of life. They are used as building blocks of membranes, cells and tissues, as energy sources, either immediate or stored, as precursors to a variety of other bio-molecules, as well as biochemical signals. In all biochemical processes lipids have an important role.

Many lipids, and especially triglycerides, are consumed in the human nutrition on a daily basis. In most cases, these lipids are metabolized and used for energy storage, precursors for biosynthesis of other lipids or bio-molecules. Whatever the fate of the lipids in the metabolic pathways, during and after their consumption, they interact with other nutrients or their metabolic products.

In human milk, and in most infant formulas, about 50% of the dietary calories are supplied to newborns as fat. More than 98% of this milk fat is in the form of triglycerides, which contain saturated and unsaturated fatty acids esterified to glycerol.

Fatty acids in human milk fat have a highly specific positional distribution on the glycerol backbone. This specific configuration is known to have a major contribution to the efficiency of nutrient absorption.

Palmitic acid (C16:0) is the predominant saturated fatty acid, constituting 20-25% of the fatty acids in mature human milk. 70-75% of this fatty acid are esterified at the *sn*-2 position of the triglycerides. In contrast, palmitic acid present in vegetable oils, which are most commonly used in the manufacture of infant formulas, is esterified at the *sn*-1 and *sn*-3 positions, while the *sn*-2 position is predominantly occupied by unsaturated fatty acids.

Triglyceride digestion by the infant

The triglyceride digestive process of the neonate is complex. It is initiated by a gastric phase catalyzed by gastric or lingual lipase [Hamosh M. (1990) *Nutrition*; **6**:421-8]. This initial lipolysis allows maximal activity of pancreatic colipase-dependent lipase during the intestinal phase of digestion. The pancreatic lipase system attacks the triglyceride with a high degree of positional specificity. Lipolysis occurs predominantly at the *sn*-1 and *sn*-3 positions, yielding two free fatty acids and a 2-monoglyceride [Mattson FH. & Beck LH. (1956) *J. Biol. Chem.*; **219**:735-740]. Monoglycerides are well absorbed independent of their constituent fatty acid. In contrast, the absorption of free fatty acids varies greatly, depending on their chemical structure. Mono and polyunsaturated fatty acids are well absorbed, as are saturated fatty acids of 12 carbons or less in chain length. The coefficient of absorption of free long chain saturated fatty acids i.e. palmitic acid is relatively low [Jensen C, *et al.* (1988) *Am. J. Clin. Nutr.*; **43**:745-51], due in part to a melting point above body temperature (~63°) and the tendency of these fatty acids to form hydrated fatty acid soaps with minerals such as calcium or magnesium at the pH of the intestine [Small DM. (1991) *Annu. Rev. Nutr.*; **11**:413-434].

Several studies have demonstrated the preferential absorption of palmitic acid when present at the triglyceride *sn*-2 position [Lien EL. *et al.* (1997) *J. Ped. Gastr. Nutr.*; **52**(2):167-174; Carnielli VP. *et al.* (1995) *Am. J. Clin. Nutr.*; **61**:1037-1042; Innis SM. *et al.* (1993) *Am. J. Clin. Nutr.*; **57**:382-390; Filer L.J. *et al.* (1969) *J. Nutr.*; **99**:293-8]. Studies comparing the palmitic acid absorption of human milk and formulas conclude that the absorption of palmitic acid is higher in human milk [Chappel JE. *et al.* (1986) *J. Pediatr.*; **108**:439-447; Hanna FM. *et al.* (1970) *Pediatr.*; **45**:216-224; Tommarelli RM, *et al.* (1968) *J. Nutr.*; **95**:583-90]. The greater absorption of fat and calcium in breast-fed infants compared with those fed formula has been ascribed to two factors: the presence in breast milk of a lipolytic enzyme (the bile salt-stimulated lipase) and the relatively high proportion of palmitic acid at the *sn*-2 position of the triglyceride [Hernell O. *et al.* (1988) *Perinatal Nutrition.* New York: Academic Press.; 259-272; Wang CS. *et al.* (1983) *J. Biol. Chem.*; **258**:9197-9202]. Higher palmitic acid absorption was obtained with formulas rich in palmitic acid esterified in the *sn*-2 position of the triglycerides, than with those containing palmitic acid predominantly esterified in the *sn*-1,3 positions [López-López A. *et al.* (2001) *Early Hum. Dev.*; **65**:S83-S94].

A study comparing the absorption of fat and calcium by infants fed a formula containing a blend of palm olein and soy oil (high levels of palmitic acid at the *sn*-1,3 positions) and a formula containing a blend of soy oil and coconut oil (low levels of palmitic acid) showed that the mixture of palm olein and soy oil, although providing the proportion of palmitic and oleic acids similar to those of human milk fat, was less absorbed [Nelson SE. *et al.* (1996) *Am. J. Clin. Nutr.*; **64**:291-296]. Another study showed that fat absorption in infants fed formula containing lard was reduced when the high proportion of *sn*-2 palmitin in lard was reduced to 33% by chemical randomization [Filer (1969) *id ibid.*].

The composition of monoglycerides absorbed from the intestinal lumen is important to the fatty acid distribution of circulating lipids because about 70% of the fatty acids absorbed as *sn*-2 monoglycerides are conserved in the original position during re-esterification to form triglycerides in the intestinal cells [Small (1991) *id ibid.*].

Studies in piglets provided evidence that palmitic acid, when absorbed from milk or formula with rearranged triglycerides as a *sn*-2 monoglyceride, is conserved through the process of triglyceride reassembly in the enterocyte and secretion in plasma lipoprotein triglycerides [Innis SM. *et al.* (1995) *J. Nutr.*; **125**:73-81]. It has also been shown that the distribution of saturated fatty acids in human milk and infant formula is a determinant of the fatty acid distribution of infant plasma triglycerides and phospholipids [Innis SM. *et al.* (1994) *Lipids.*; **29**:541-545].

During the first year of life an infant's birth weight triples and the length is increased by 50%. To meet the requirements of their rapidly expanding skeletal mass, growing infants require a bioavailable source of calcium. For formula-fed infants, availability of calcium depends on the composition of the formula [Ostrom KM. *et al.* (2002) *J. Am. Coll. Nutr.*; 21(6):564-569].

As mentioned above, the digestion of triglycerides involves lipolysis at the *sn*-1 and 3 positions and formation of free fatty acids and 2-monoglycerides. When palmitic acid is located at the *sn*-1,3 positions, as is the case in most infant formulas, it is released as free fatty acid which tends to form insoluble calcium soaps. In contrast, palmitic acid esterified to the *sn*-2 position, as in human milk, is unavailable to form calcium soaps [Small (1991) *id ibid.*].

Several studies have shown a correlation between formulas containing high levels of palmitic acid situated at the *sn*-1,3 positions of the triglyceride and reduction in calcium absorption [Nelson SE. *et al.* (1998) *J. Amer. Coll. Nutr.*;

17:327-332; Lucas A. *et al.* (1997) *Arch. Dis. Child.*; 77:F178-F187; Carnielli VP. *et al.* (1996) *J. Pediatr. Gastroenterol. Nutr.* 23:553-560; Ostrom (2002) *id ibid.*; Hanna (1970) *id ibid.*]. In addition, it was shown that dietary triglycerides containing palmitic acid predominantly at the *sn*-2 position, as in human milk, have significant beneficial effects on the intestinal absorption of fat and calcium in healthy term infants as well as in preterm infants [Carnielli (1996) *id ibid.*; Carnielli (1995) *id ibid.*; Lucas (1997) *id ibid.*]. Infants fed a formula containing high levels of palmitic acid at the *sn*-1,3 positions showed greater fecal excursion of calcium and, hence, a lower percentage absorption of calcium compared to infants fed a formula containing low levels of palmitic acid [Nelson (1996) *id ibid.*]. Fecal excretion of calcium was closely related to the fecal excretion of fat. This study also showed that urinary phosphorus excretion increased and phosphorus retention decreased when infants were fed the formula containing high levels of palmitic acid at the *sn*-1,3 positions. These findings presumably reflect lower availability of calcium for deposition in bones.

Another important issue which is associated with formula feeding is constipation in both term and preterm infants which, in the latter, can lead to life threatening complications. By contrast, constipation is rare in breast fed term infants. A study comparing breast fed and formula fed infant stool hardness and composition showed that calcium fatty acid soaps are positively correlated to stool hardness. Stools from formula-fed infants were significantly harder than those of the breast-fed infants suggesting different handling of saturated fatty acids [Quinlan PT. *et al.* (1995) *J. Pediatr. Gastr. and Nutr.*; 20:81-90].

In an attempt to overcome the decreased calcium absorption and hard stool phenomena, infant formula manufacturers tend to deviate from the fatty acid profile by replacing palmitic acid with lauric acid and, in some cases, by increasing the polyunsaturated fatty acid content. Studies have shown that

fatty acid composition of the diet influences the fatty acid composition of developing infant tissue [Widdowson E.M. (1975) *Br. Med. J.*; 1:633-5; Carlson SE. *et al.* (1986) *Am. J. Clin. Nutr.*; 44:798-804; Innis SM. *et al.* (1990) Am. J. Clin. Nutr.; 5:994-1000; Koletzko B. *et al.* (1989) *Eur. J. Pediatr.*; 148:669-75] and thus the lipoprotein and lipid metabolism differ between breast-fed and formula-fed infants [Putnam J.C. *et al.* (1982) *Am. J. Clin. Nutr.*; 36:106-114; Innis SM. *et al.* (1992) *Am. Coll. Nutr.*; 11:63S-8S; Van Biervliet JP. *et al.* (1981) *Acta. Paediatr. Scand.*; 70:851-6].

Innis and colleagues [Innis (1993) *id ibid.*], when comparing three formulas containing similar amounts of saturated fatty acids - C8-C14, C16 from palm oil (predominantly in the *sn*-1,3 positions), or C16 from synthesized triglyceride (predominantly in the *sn*-2 position) - showed that the chain length of saturated fatty acids in infant formula influences the metabolism of the dietary oleic, linoleic and alpha-linolenic acids. This study also showed that the *sn*-2 configuration of C16 in human milk triglycerides seems to have unique properties that extend beyond absorption. These include effects on HDL and cholesterol concentrations, and the cholesterol ester fatty acid composition.

The impact of soap formation on calcium absorption can be significant. Many infant formulas contain sufficient saturated fatty acids to form soaps with virtually all the calcium available.

US Patent No. 4,876,107 (corresponding to EP 0 209 327) describes a substitute milk fat composition which is suitable as replacement fat in infant formulations. In this fat composition the total palmitic acid residues present is as high as 45%, with at least half of the fatty acid residues at the 2-position of the glycerol backbone being palmitic. The product has about 27% palmitic acid residues at the 1- and 3-positions, and the other substituents at the 1- and 3-positions are mainly unsaturated C_{16} and C_{18} fatty acid moieties. The

fat composition is prepared by a specific process, in the presence of Hexane. Rather high levels of the fat compositions are required for the preparation of final infant formulations.

EP 0 495 456 also discloses substitute milk fat compositions. These compositions have a saturated fatty acid content at the *sn*-2 position of at least 40%, most of which palmitic acid residues, and contain 0.2-7% linolenic acid moieties, 70% of which are bonded at the 1- and 3-positions of the glycerol moieties, the remaining acid moieties at the 1- and 3-positions, other than unsaturated fatty acids, are saturated C_4-C_{12} fatty acids.

US Patent No. 5,658,768 discloses a multiple-step process for preparing triglyceride compositions in which more than 40% of the saturated fatty acid moieties are at the 2-position. Many of the steps involve enzymatic modifications.

In sum, one of the most pronounced differences between mother's milk and infant formulas is in the fat composition. In mother's milk, most of the saturated fatty acids (about 70%, mainly palmitic acid) are located at the *sn*-2 position of the triglycerides while the *sn*-1,3 positions are mainly occupied with unsaturated fatty acids. However, most infant formulas do not contain such composition and the result is the loss of energy (in the form of palmitic acid) and calcium by the infants. The reason for that is first and foremost, the limited availability of a fat mimicking the human breast milk fat. Currently, there is yet no natural alternative from a safe vegetal source. Limited sources are those of animal origin, which are extremely non-safe in a most delicate field like infant nutrition. One alternative in the past was to use lard, however health risks related to porcine viruses that can be transmitted to infants have caused this fat source to be eliminated. While there exist commercially available fats which mimic the fat composition of human breast

milk, such as those described, e.g. in EP 0 209 327, they suffer several major drawbacks, inter alia the following:

- Good blends are of very high cost and apparently limited availability, due to inferior methods of production. This is even more pronounced if the blends are to be used together with other new and relatively costly important nutrients, such as long-chain polyunsaturated fatty acid (LC-PUFA);
- Commercial versions available on the market are inferior in terms of health benefits (only 43% of the total palmitic acid residues are esterified at the *sn*-2 position). A ratio of less than 50% (of the total palmitic acid is esterified at the *sn*-2 position) may have no meaningful benefits in terms of calcium and energy intake.
- Production is by using a genetically modified enzyme, hence the product may be considered as GMO with the risks involved.
- The products have to be incorporated to the formula blends at relatively high quantities, which may leave little room for any additional important oils and lipids to be incorporated without raising the total fat content of the formula.

Therefore, there are three important points when it comes to the triglyceride composition of human milk fat replacement:

1) The total amount of palmitic acid;
2) The ratio of palmitic acid at the *sn*-2 position (expressed as percent of palmitic acid at the *sn*-2 position from the total palmitic acid level);
3) The amount of oleic acid.

The amount of oleic acid is important in order to preserve the calcium and energy for the infant, and ensure normal and healthy development, since the fatty acids at the *sn*-1,3 positions of the oil component should be unsaturated. The higher the amount of unsaturated fatty acids, such as oleic acid, the better, since this indicates that most of the *sn*-1,3 positions are occupied by

fatty acids that will not create harmful complexes with calcium. Consequently, the infant will not lose either energy (in the form of fatty acids) or calcium.

In order to find an optimal infant formula, wherein the amounts and composition of the fats are as close as possible to mother's milk, which would also be cost-effective, the present inventors have developed a new fat-based preparation in which the amount of palmitic acid residues at the *sn*-2 position of the triglycerides, and the amount of oleic acid are as close as possible to the optimum desired, as described below.

Thus, it is an object of the present invention to provide compositions typically comprising the fatty acids palmitic, oleic, linoleic and stearic acid, wherein up to 70% of the palmitic acid present is located in the *sn*-2 position. The invention also provides the process for preparation of said composition. Other uses and objects of the invention will become clear as the description proceeds.

Summary of the Invention

The present invention relates to an enzymatically prepared fat base composition comprising a mixture of vegetable-derived triglycerides, characterized in that it has a total palmitic acid residues content of at most 38% of the total fatty acid residues, and in that at least 60%, preferably 62% of the fatty acid moieties at the *sn*-2 position of the glycerol backbone are palmitic acid residues.

In the fat base composition of the invention, preferably at least 70% of the fatty acid moieties at the *sn*-1 and *sn*-3 positions of the glycerol backbone are unsaturated. More preferably, at least 40%, preferably 40-60%, of the unsaturated fatty acid moieties at the *sn*-1 and *sn*-3 positions are oleic acid

moieties. Particularly, at least 6%, preferably 6-17%, of said unsaturated fatty acid moieties at the *sn*-1 and *sn*-3 positions are linoleic acid moieties.

The invention further relates to a substitute human milk fat composition comprising a blend of at least 25% of the fat base composition of any one of claims 1 to 5 with up to 75% of at least one vegetable oil.

The vegetable oil may be selected from the group comprising soy oil, palm tree oil, canola oil, coconut oil, palm kernel oil, sunflower oil, corn oil and rapeseed oil.

In a further aspect, the invention relates to an infant formula comprising the substitute human milk fat composition of the invention. The infant formula of the invention may optionally further comprise vitamins, minerals, nucleotides, amino acids and carbohydrates.

In yet another embodiment, the invention relates to a process for the preparation of the fat base composition of the invention, comprising essentially the steps of reacting a palmitic acid rich oil with unsaturated fatty acids, preferably oleic acid, in the presence of an insoluble catalyst; removing the catalyst; distilling the excess free fatty acids; bleaching the oil; and optionally deodorization of the resulting product. The process of the invention may optionally further comprise a step of fractionation before the deodorization step.

Still further, the invention relates to a process for the preparation of the substitute human milk fat composition of the invention, comprising admixing said vegetable oil with the fat base composition of the invention.

Also encompassed are the use of the fat base composition of the invention in the preparation of a substitute human milk fat composition for infant formulae, and its use in the preparation of an infant formula.

Detailed Description of the Invention

In an attempt to provide the best and closest to the mother's human milk fat substitute, the present inventors have generated novel fat compositions in which the amounts and positions of saturated and unsaturated fatty acids have been manipulated so as to achieve that goal.

The terms "fat" and "lipid" are used herein interchangeably.

Lipids, under the scope of this invention, include triglycerides and derivatives, such as mono- and di-glycerides.

Preferably, the lipid constituent of the dietary ingredient of the invention is based on a synthetic oil (which can be produced both chemically and, preferably, enzymatically) which mimics the triglyceride composition of human breast milk fat. This oil has, preferably, a high level of palmitic acid at the *sn*-2 position of the triglycerides, consisting of above 40%, and preferably over 60%, more preferably over 65% of the total palmitic acid content. Furthermore, this oil has a high level of unsaturated fatty acids at *sn* positions 1 and 3, preferably over 50%. This ingredient is also referred to herein as **In***Fat*™ (Enzymotec Ltd., Migdal HaEmeq, Israel).

Thus, in a preferred embodiment, the present invention provides an enzymatically prepared fat base composition comprising a mixture of vegetable-derived triglycerides, characterized in that:

- the total palmitic acid residues content is at most 38% of the total fatty acid residues;

- at least 60% of the fatty acid moieties at the *sn*-2 position of the glycerol backbone are palmitic acid residues.

InFat is an advanced fat-base ingredient for the production of fat preparations used in infant nutrition formulas. It is an exclusive fat-base, designed and manufactured with specific triglycerides composition and structure.

The essential features of the fat-base composition are as follows:
- at least 62% of the total palmitic acid residues are at the *sn*-2 position of the glycerol backbone;
- at least 70% of the fatty acid moieties at the *sn*-1 and *sn*-3 positions of the glycerol backbone are unsaturated;
- at least 40%, preferably 40-60%, of said unsaturated fatty acid moieties at the *sn*-1 and *sn*-3 positions are oleic acid moieties;
- at least 6%, preferably 6-17%, of said unsaturated fatty acid moieties at the *sn*-1 and *sn*-3 positions are linoleic acid moieties.

InFat is designed to have the right amount of palmitic acid and at the correct position of the triglycerides. The unique composition and structure of InFat mimics the fat composition and properties of human breast milk fat, and when incorporated in infant nutrition, offers exceptional nutritional and developmental benefits for infants and babies. This fat ensures optimal intake of calcium and also energy, in the form of free fatty acids.

In another aspect, the present invention provides a substitute human milk fat composition comprising a blend of at least 25% of the fat base composition of the invention, with up to 75% of at least one vegetable oil. This means that the fat base composition of the invention may be used to prepare a blend of substitute human milk fat, at a ratio of 1 part of the fat base composition to 3 parts of one or a combination of vegetable oil/s. In the following Examples,

five blends are presented, InFat 1, InFat 2, InFat 3, InFat 4 and InFat 5, wherein different amounts of the fat base composition (InFat) were used, from 30% up to 83% of the content of the blend.

Thus, the present invention also provides a dietary ingredient comprising an edible lipid, wherein said edible lipid is a mimetic substitute of human breast milk fat.

InFat is designed to be blended with other complementary oils in order to achieve the final specified fatty acids composition of the infant formula. The right amount of palmitic acid, which is designed according to the structure and properties of human breast milk fat, offers not just better nutrition for infants but also greater flexibility when blending with complementary oils.

In this manner, the substitute human milk fat composition, i.e. the blend, may be prepared with any one or a combination of, for example, the following vegetable oils: soy, palm tree, canola, coconut, palm kernel, sunflower, corn and rapeseed oil, as well as other vegetable oils and fat.

Most importantly, the substitute human milk fat composition may be used in the preparation of infant formula.

Hence, in a further aspect, the present invention provides an infant formula, comprising the substitute human milk fat composition as described above. The infant formula provided by the invention is comprised of at least one protein component and at least one fat component, wherein said fat component is the substitute human milk fat composition as described above, and further optionally comprises vitamins, minerals, nucleotides, amino acids and carbohydrates.

In a yet further aspect, the present invention provides a process for the preparation of the fat base composition of the invention, comprising the steps of:

(a) reacting a palmitic acid rich oil with unsaturated fatty acids, preferably oleic acid, in the presence of an insoluble catalyst;

(b) removing the catalyst;

(c) distilling the excess free fatty acids;

(d) bleaching the oil; and

(e) optionally deodorizing the resulting composition.

This reaction is carried out at temperatures of preferably between 50°C and 60°C.

In order to enhance the quality of the fat base, an optional further step of fractionation may precede the deodorization step (e).

The enzyme used in the above method is a 1,3 regio-specific lipase, which is preferably immobilized and surfactant coated. This enzyme preparation can be prepared according to the technology developed by the present inventors, and described in WO99/15689.

In EP 0 209 327 referred to above, for example, the process for preparing the fat composition involves the use of hexane, and a further step for its removal An important advantage of the process described herein is that it does not involve the use of solvents, which may leave potential toxic residues in the fat composition to be used in the preparation of infant formulas. Thus, the process of the invention yields a safer product.

In yet another aspect, the present invention provides a process for the preparation of the substitute human milk fat composition of as described herein, comprising admixing a vegetable oil or combination of oils with the

fat-base composition of the invention. As mentioned above, several vegetable oils may be used for preparing the composition, including soybean oil, palm tree oil, canola oil, coconut oil, palm kernel oil, sunflower oil, corn oil and rapeseed oil.

The present invention is defined by the claims, the contents of which are to be read as included within the disclosure of the specification.

Disclosed and described, it is to be understood that this invention is not limited to the particular examples, process steps, and materials disclosed herein as such process steps and materials may vary somewhat. It is also to be understood that the terminology used herein is used for the purpose of describing particular embodiments only and not intended to be limiting since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The following Examples are representative of techniques employed by the inventors in carrying out aspects of the present invention. It should be appreciated that while these techniques are exemplary of preferred embodiments for the practice of the invention, those of skill in the art, in light

of the present disclosure, will recognize that numerous modifications can be made without departing from the spirit and intended scope of the invention.

Examples

Example 1: Preparation of InFat

Specifically, InFat is an oil containing over 90% triglycerides. In*Fat* also contains diglycerides. In some formulations, In*Fat* can include up to 3% free fatty acids. The triglycerides of this product are characterized by a high percentage of palmitic acid at the *sn*-2 position, over 60%, preferably over 65% of the total palmitic acid in this oil. The *sn*-1 and 3 positions are characterized by a high percent of oleic acid and other unsaturated fatty acids.

InFat is produced by reacting a mixture of triglycerides, rich in palmitic acid, preferably above 78%, with a mixture of free fatty acids rich in oleic acid, preferably above 75%, with a low content of palmitic and stearic acids, preferably below 6%.

Preferably, the triglyceride mixture is produced from double-fractioned palm stearin and the free fatty acids (FFA) mixture is obtained from palm kernel oil after fractionation, or from high oleic sunflower oil. The ratio between triglycerides and FFA is from about 1:1 to about 1:10, particularly 1:4. The two mixtures are blended in stirred (optionally large scale) reactors with no additional solvent. To this mixture is added a surfactant coated immobilized 1,3-lipase (prepared as described in Applicant's WO00/56869), using an insoluble ion exchange resin for the immobilization and a suitable 1,3 lipases as described in said WO00/56869, preferably *Rhizopus oryzae* lipase. The mixture of triglycerides, FFA and catalyst is stirred at 50-60°C for about 3-9 hours, to yield the final and desired triglycerides mixture. Progress and endpoint are monitored by positional analysis of triglycerides. The final process mixture is separated from the catalyst by decantation or filtration and

the mixture of triglycerides and excess FFA are distilled to remove the FFA. FFA removal can be achieved, inter alia, by steam stripping or by molecular distillation. The distilled FFA are contaminated with palmitic acid, released from the triglyceride raw material during the reaction. The FFA can be purified from the excess palmitic acid in order to be reused in the reaction stage by several processes including selective dry fractionation, or fractional distillation. The triglyceride product is further treated in order to improve color, odor and taste with bleaching and deodrization stages. Optionally, the product is fortified with natural antioxidants to increase the shelf life of the product. The catalyst can be further recycled, to be re-used in further batches. A single catalyst preparation can be used for more than 100 batches (ratio of about 1:2 catalyst:triglycerides in the batch) and 1MT of catalyst is enough to produce more then 200 MT of final product. The product can be also produced by using a fixed bed reactor and a continuous process.

The following Table 1 details the contents of the resulting fat base composition of the invention (InFat), also referred to as "the concentrate material".

Example 2: Preparation of InFat Blends

InFat1 blend: InFat1 blend was produced by mixing several vegetable oils to a final fatty acid composition and palmitic acid positional distribution according to the specification below. The required vegetable oils and fats (all formulation components except the InFat) are mixed together and optionally are randomized to obtain 33% of the palmitic acid esterified in the 2nd position. Afterwards, the interesterified blend is simply mixed with the InFat in the selected ratio. For InFat1 blend the following were used: 30% InFat concentrate (see Table 1 for fatty acid composition), 23% Coconut oil, 21% Palm oil, 10% Corn oil, and 16% Rapeseed oil. All vegetable oils used are standard food grade oils.

Thus, this blend is achieved with only 30% of InFat (the concentrate material). The ratio of *sn*-2:total palmitic acid is approximately 48.7%, and total palmitic acid is 22.8%. Even this blend, the simplest of blends presented herein, and containing only 30% of the InFat concentrate, is superior to available commercial HMF equivalent (approx. 43% ratio).

InFat2 blend: This blend was prepared in a similar manner, using 50% InFat concentrate (see Table 1 for fatty acid composition), 15% Coconut oil, 15% Palm oil, 5% Sunflower oil, 10% Corn oil, and 5% Rapeseed oil. All vegetable oils used are standard food grade oils.

Thus, this blend uses 50% of InFat™. The ratio of *sn*-2:total palmitic acid is approximately 56.3% and total palmitic content of 25.4% (25.7% in HMF) (this is superior to the ratio obtained with a similar percentage of available commercial HMF equivalent (approx. 52.5% ratio)).

InFat3 blend: This blend was prepared in a similar manner, using 63% InFat concentrate (see Table 1 for fatty acid composition), 16% Coconut oil, 9% Palm oil, and 12% Corn oil. All vegetable oils used are standard food grade oils.

The total palmitic acid is closer to breast milk, while only 63% of InFat were introduced. The ratio of *sn*-2:total palmitic acid is approximately 60.6%.

InFat4 blend: This blend was prepared in a similar manner, using 73% InFat concentrate (see Table 1 for fatty acid composition), 13.5% Coconut oil and 13.5% Rapeseed oil. All vegetable oils used are standard food grade oils.

This blend uses 73% of InFat™. The ratio of *sn*-2:total palmitic acid is approximately 67.4%, and total palmitic acid content is 25.1% (this is

superior to the ratio obtained with a similar percentage of available commercial HMF equivalent (approx. 62.3 or 62.7% ratio)).

InFat5 blend: This blend was prepared in a similar manner, using 83% InFat concentrate (see Table 1 for fatty acid composition), 9.3% Coconut oil and 7.7% Sunflower oil. All vegetable oils used are standard food grade oils.

This is a very superior blend, in that it is similar to breast milk in the ratio of *sn*-2:total palmitic acid (68.5% vs. ~70% in HMF), total C16:0 (27.7% vs ~26% in HMF) and *sn*-2 C16:0 (56.9% vs. 57% in HMF).

The compositions of these five blends (InFat1, InFat 2, InFat 3, InFat 4, and InFat 5) are also given in Table 1.

Table 1

	InFat	InFat 1	InFat 2	InFat 3	InFat 4	InFat 5	Milk Fat
Fatty acid							
C12		11.1	7.2	7.8	6.5	4.4	2.3
C14		4.5	3.1	3.3	2.8	2.1	5
C16	32	22.8	25.4	26.9	25.1	27.7	25.7
2nd C16	67.2	33.4	42.9	48.9	50.8	56.9	57.5
ratio	70.0	48.7	56.3	60.7	67.4	68.5	74.6
C16:1							
C18	4	2.3	3.0	3.1	3.5	4.0	7.1
C18:1	53.1	38.4	40.8	41.6	47.9	46.6	38.5
C18:2	8	13.5	15.6	12.8	8.6	11.7	11.7
C18:3		1.7	0.6				
% concentrate	100	30	50	63	73	83	
Coconut oil		23	15	16	13.5	9.3	
Palm Kernel Oil							
Palm oil		21	15	9			
Sunflower			5			7.7	
Corn oil		10	10	12			
Rapeseed		16	5		13.5		
Soybean							
Total	100	100	100	100	100	100	

The table shows the fatty acid composition of the InFat concentrate and the InFat blends 1-5 as compared to HMF. C16 represents the total palmitic acid content. 2nd C16 represents the % palmitic acid of total *sn*-2 position fatty acids. The ratio means the % of *sn*-2 palmitic acid of total palmitic acid normalized per position {(% of *sn*-2 palmitic)/(3x%total palmitic acid)}x100. All numbers represent %(w/w), except the ratio which is defined as %.

Example 3: Comparison of InFat blends to commercially available fat concentrates

Table 2 is a comparison of InFat blends described in Example 2 to commercially available HMF mimetic preparations. In particular, it is important to compare the composition of InFat (the concentrate, Table 1) with that of the two commercially available concentrates (Concentrates 1 and 2, Table 2), and to compare the various InFat blends (InFat1-5, Table 1) with Blends 1-4 of the commercial concentrates (Table 2).

Comparison of the two concentrates of Table 2 with the InFat concentrate of Table 1 reveals that InFat has lower palmitic acid content which is closer to HMF, the sn-2 palmitic acid level is also lower and closer to HMF, and the ratio is higher and closer to the ratio in HMF. It should be noted that the concentrates are not usually used "as is" in infant formulas, since they do not contain other fatty acids required for the infant nutrition such as medium and short chain fatty acids, as well as LC-PUFA, such as Omega-3 DHA and Omega-6 ARA. The incorporation of such fatty acids is obtained by different blends.

As already described above, the blends of InFat are also superior to the blends of Table 2 in terms of mimicking HMF as well as in the proportions of the concentrate needed to obtain each blend, keeping in mind that the concentrate, being a synthetic oil, is the major-cost component of the blend and hence should be kept to a minimum in order to achieve cost effectiveness of such a nutrition product.

As described above, InFat2 can be compared to blend 2, which also utilizes a 50% concentrate. In InFat2 the ratio of *sn*-2:total palmitic acid is approximately 56.3% (57.5% in HMF) and total palmitic content of 25.4% (25.7% in HMF). Blend 2 of Table 2 also uses 50% of concentrate but an

inferior ratio of only 52.5% is obtained. Furthermore, in both total palmitic and *sn*-2 palmitic, InFat has some advantage in terms of similarity to HMF.

InFat3 blend has a ratio similar to blends 1, 3 and 4 of Table 2 but utilizes only 63% of concentrate, while these blends of Table 2 utilize 70%. InFat3 is also superior in terms of total palmitic acid which is closer to HMF.

InFat4, which is based on 73% InFat concentrate, can be compared to blends 1, 3 and 4 of Table 2, which are all also based on 70% of a commercial concentrate. In InFat4, the ratio of *sn*-2:total palmitic acid is approximately 67.4%, and total palmitic acid content is 25.1%, both values in good accord with HMF. Blends 3 and 4 of Table 2 have ratios of 62.8% and 62.3%, inferior to the present example, and a total of 30% or 30.5% palmitic acid, which is higher than in HMF.

InFat5 of course is superior and is not met by any of the blends described in Table 2.

Table 2

	Blend 1	Blend 2	Blend 3	Blend 4	Concen-trate 1	Concen-trate 2	Milk Fat
C12	9.5	5	5	10			2.3
C14	3	2	1.5	3			5
C16	33	26	30	30.5	44.5	40	25.7
2nd C16	57	41	56.5	57	80	80	57.5
Ratio	57.6	52.6	62.8	62.3	59.9	66.7	74.6
C16:1	2	1.5		2.5	3.5	3.5	5.1
C18	5.5	5	1	5	6	6.5	7.1
C18:1	35	33.5	47.5	36.5	41.5	44.5	38.5
C18:2	10.5	23	15	10.5	4.5	5.5	11.7
C18:3							
% concen-trate	70	50	70	70	100	100	
Coconut oil							
Palm Kernel Oil	20	10	10	20			
Palm oil							
Sun-flower	10	10	20	10			
Corn oil							
Rapeseed							
Soybean		30					
Total	100	100	100	100	100	100	0

Example 4

Infant formula based on In*Fat*

An infant formula comprising In*Fat* and additional oils and fats that mimic the human breast milk fat composition is prepared as follows: required oil blend is prepared by mixing of a selected formulation (e.g. those of Table 1). The oil is mixed together with the other infant formula components (proteins, carbohydrates, minerals, vitamins and others). The slurry is passed through a pressure homogenizer to get a stable emulsion. Homogenized product is then dried in a spray drier to obtain the final product. Other additives may be added to the dry powder to obtain final formulation.

The fat fraction produced by the blending of InFat with other oils and fats as described above is further blended with other nutrients such as proteins, minerals, vitamins and carbohydrates to yield a food product supplying the

infant with the major nutrients also found in human milk. The nutrients and fats are homogenized using pressure homogenization and spray dried to yield a homogenous powder. The powder is further re-dispersed in water (approx. 9g powder per 60 ml water) to yield a ready-to-feed formula. The fat content of the ready feed is approx. 3.5 g per 100 ml which corresponds to the fat content of human breast milk, which is in the range of 30-40g/L.

The fatty acid composition of a blend of InFat (30%) with other oils and fats used to create an infant formula is as follows:

Fatty acid	**%**
C10:0	1.3
C12:0	10.3
C14:0	4.3
C16:0	23.5
sn-2 C16:0 (% of total C16:0)	43
C18:0	3.2
C18:1	39.2
C18:2	13.6
C18:3	1.7
C20:0	0.3
C20:1	0.3
C22:0	0.2

	per 100 g powder	Per 100 ml ready to feed
Energy kcal	508	68
Sodium mg	140	18.8
Protein g Lacatalbumin/Casein 60/40)	11.4	1.5
Fat g	26.5	3.5
Saturated fat g	14.5	1.95
Linoleic acid	5000	670
Alpha-linolenic acid mg	530	71
Arachidonic acid mg	115	15.3
Docosahexaenoic acid mg	108	14.4
Cholesterol mg	2	0.3
Lactose g	56	7.5
Calcium mg	430	57.3
Phosphorus mg	250	33.5
Potassium mg	420	56.3
Chloride mg	300	40.2
Iron mg	5.25	0.7
Magnesium mg	50	6.7
Zinc mg	3.5	0.47
Copper mcg	300	40.2
Manganese mcg	45	6
Iodine mcg	45	6
Taurine mg	45	6
Vitamin A I.U.	1500	200
Vitamin D I.U.	300	40.2

Vitamin E mg	10	1.3
Vitamin K mcg	45	6
Vitamin C mg	60	8
Vitamin B_1 mcg	400	53
Vitamin B_2 mcg	800	127
Vitamin B_6 mcg	375	50
Vitamin B_{12} mcg	1.15	0.2
Niacin mg	6	0.8
Panthothenic acid mg	3	0.4
Folic acid mcg	67	9
Biotin mcg	14.3	1.9
Choline mg	37.5	5
Inositol mg	22.5	3
Moisture %	3	

The level of fat and the exact composition can be controlled in order to yield infant formulas designed to mimic the different lactation periods.